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ETHANOL TEST PACKAGE
(CAS RN 64-17-5)

TEST PLAN
TEST PLAN JUSTIFICATION
ROBUST SUMMARY

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Sponsored by
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Test Plan

Ethanol	Information?	GLP or OECD?	Acceptable?	Test?
Physical-Chemical Data				
Melting point	Y	N	Y	N
Boiling point	Y	N	Y	N
Vapor pressure	Y	N	Y	N
Partition coefficient	Y	N	Y	N
Water solubility	Y	N	Y	N
Environmental Fate				
Photodegradation	Y	N	Y	N
Stability in water	Y	N	Y	N
Transport between compartments	Y	N	Y	N
Biodegradation	Y	N	Y	N
Ecotoxicity				
Acute toxicity to fish	Y	N	Y	N
Acute toxicity to aquatic plants	Y	N	Y	N
Acute toxicity to aquatic invertebrates	Y	N	Y	N
Health Endpoints				
Acute toxicity to mammals	Y	N	Y	N
Genetic toxicity in vivo	Y	N	Y	N
Genetic toxicity in vitro	Y	N	Y	N
Repeat dose toxicity	Y	Y	Y	N
Reproductive toxicity	Y	N	Y	N
Developmental toxicity	Y	N	Y	N

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Test Plan Justification

This document summarizes findings of the robust summary for ethanol, describes other relevant literature for ethanol, and explains why no additional testing for ethanol is proposed.

1. Physical-chemical properties

Next to water, ethanol is perhaps the most-used solvent in chemistry and biology, The boiling and melting points, vapor pressure, water solubility, and partition coefficient of ethanol are well known and can be found in standard texts. Original papers documenting these properties are not readily available in all cases, since the properties were documented so long ago. Although summaries for these properties lack certain desired information, no additional testing is proposed.

2. Environmental fate

Some data regarding photodegradation of ethanol was located and is included in the robust summary. No experimental data ***per*** se on ethanol's stability in water were located, but it is common scientific knowledge (as well as common knowledge) that ethanol is stable in water over years or centuries, as attested to by the longevity of alcoholic beverages. The argurnent is made, based on reactivity of functional groups, that ethanol does not undergo hydrolysis in a meaningful sense. Fugacity of ethanol was modeled using the EQC model, as recommended by HPV Challenge guidance. Biodegradation of ethanol is described in two papers in the robust summary. Ethanol is widely recognized as being readily biodegraded in the environment, as it is both a metabolite of and nutrient for microbes. This subject was recently reviewed by Ulrich (1999). No additional testing on the environmental fate of ethanol is proposed at this time.

3. Ecotoxicity
    a. Acute toxicity to fish

Four studies were found giving LC_{50}'s for rainbow trout and fathead minnows over 24 and/or 96 hours. One study was conducted by an EPA laboratory and another by a national fisheries laboratory. The results are consistent, indicating lethal concentrations in excess of 11,000 mg/l. Similar lethal concentrations are cited in the Hazardous Substances Databank record for ethanol: 14,200 mg/l and 15,300 mg/l at 96 hours for fathead minnows, Thus, while some study parameters are missing from the summarized reports, the database is considered adequate at the screening level, and no further testing is needed.

b. Acute toxicity to aquatic invertebrates

From four published studies, LC_{50}'s for ethanol were identified for five species of invertebrates *(Artemia, Ceriodaphnia, Daphnia, Hyallela,* and *Palaemonetes); Daphnia* was tested over two durations, and *Artemia* at three ages, giving a total of eight LC,, values. *Artemia* was the most sensitive species tested, with LC,, values of 1,833 mg/l or less. LC_{50}'s for all other tested species were at least 5,000 mg/l. Confidence intervals are given for every LC,, determination. While the studies lack some information requested for the robust summaries, this database appears adequate at the screening level, and no further testing is needed.

c. Acute toxicity to aquatic plants

From five published studies, effect concentrations for ethanol were identified for six species of aquatic invertebrates *(Ceriodaphnia, Chlamydomonas, Chlorella, Dunaliella, Lemna [five* clones], *Selenastrum,* and *Skeletonema).* For most of these plants, ErC_{50} values were identified over four or seven days of exposure, and these values ranged from 1,000 mg/l to more than *10,000* mg/l. *Chlorella* was the most sensitive species examined. For each species except *Dunaliella,* effect levels were determined using at least four concentrations of ethanol. While the studies lack some information requested for the robust sumrnaries, this database appears adequate at the screening level, and no further testing is needed.

4. Health endpoints

a. Acute toxicity

From six published studies, a total of ten LD_{50} or LC,, tests were identified for rats and mice. Mice of both sexes were tested by oral and intraperitoneal exposure, while rats of both sexes were tested by oral exposure and males by intraperitoneal administration also. Both old and young male rats were tested by two exposure routes. In all, four strains of mice and two strains of rats were examined. No LC,, was identified as the concentrations used, 40,000-60,000 ppm, produced no deaths. Oral ethanol exposures yielded 24-hour LD_{50}'s ranging from about 5 g/kg to about 17 g/kg. The reference book, *Dangerous Properties of Industrial Materials* (1989) lists LD,, values for numerous species by several routes of exposure. The oral LD,, values therein are consistent with those presented in the robust summary for ethanol. The lowest LD,, given in the book is 963 mg/kg by the intraperitoneal route in rabbits. In addition, the Internal Agency for Research on Cancer (IARC, 1988), in its monograph on alcohol drinking, reports oral and intraperitoneal LD,, values for various species, the lowest of which was 4.3 g/kg. Given the large database on the acute mammalian toxicity of ethanol, no further testing is needed, despite minor deficiencies in the studies presented in the robust summary.

b. *In* vivo genotoxicity

The robust summary presents in vivo genotoxicity for ethanol in mice (five strains), rats, and hamsters by oral (gavage and drinking water) and intraperitoneal exposures. Endpoints examined were sister chromatid exchanges, micronuclei formation, chromosome aberrations, and dominant lethality. Three of the studies were deemed adequate for inclusion in compendia prepared by the EPA's Gene-Tox Program. The two tests in hamsters and the micronucleus test in mice were negative, but positive results were obtained in sister chromatid exchange and dominant lethality assays. The genotoxicity of ethanol was comprehensively reviewed in 1987 by Obe and Anderson for the International Commission for Protection Against Environmental Mutagens and Carcinogens. More than 30 *in vivo* tests of ethanol in animals were included, and the authors concluded that, in mammalian cells, ethanol is mostly non-genotoxic but can induce sister chromatid exchanges if metabolism is possible. IARC (1988) has also reviewed ethanol's in *vivo* genotoxicity. Despite minor deficiencies in the genotoxicity tests included in the robust summary for ethanol (also apparent in many studies not summarized), there is clearly a large and adequate database on the *in vivo* genotoxicity of ethanol. No additional testing is needed.

c. *In vitro* genotoxicity

Results of seven *in vitro* genotoxicity assays of ethanol are included in the robust summary; these studies were conducted in bacteria, yeast, Chinese hamster ovary cells, mouse lymphoma cells, and human lymphocytes. Four of the studies were deemed adequate for inclusion in various EPA Gene-Tox Program reports, and one was conducted under the auspices of the National Toxicology Program. More than 30 *in* vitro genotoxicity of ethanol were reviewed by Obe and Anderson (1987) for the International Commission for Protection Against Environmental Mutagens and Carcinogens, who concluded that ethanol *per* se generally does not induce genetic damage *in vitro* unless the test system is capable of metabolizing ethanol. IARC (1988) also reviewed ethanol's *in vitro* genotoxicity in some detail. This endpoint has been adequately tested, and no additional testing is warranted.

d. Repeated dose toxicity

The effects of chronic ethanol consumption have been tested in rats (Sprague-Dawley and Fischer 344) and mice (B6C3F 1) by the Swedish National Board of Occupational Safety and Health and the US National Toxicology Program (NTP). Both were 90-day studies, with ethanol present in liquid diets in the Swedish studies and in drinking water in the NTP studies. The Swedish studies (Holmberg *et al.*, 1986) were dose-finding efforts for a two-year carcinogenicity bioassay, while in the NTP study, ethanol was studied only as a possible modulator of urethane toxicity, as urethane is found in alcoholic beverages. Both experiments used large doses, of at least 1 g/kg-d. Elsewhere in the open literature, one can find literally hundreds of experiments in which laboratory animals were repeatedly dosed with large amounts of ethanol, usually to explore toxic endpoints recognized from the human experience, such as liver damage, central nervous system toxicity, and alcoholism, or other endpoints of interest such as hematologic or immunologic change.

Of course, the literature is also rich in data regarding the effect of alcoholic beverages (in which ethanol is the major active component) on human health. Reviews of the toxicity of ethanol or alcohol include Ahmed (1995; a broad review of the effects of ethanol), Andersson and Victorin (1996; on the toxicity of inhaled ethanol), Seitz et *al.* (1998; on the carcinogenicity of alcohol), Friedman (1998; on the cardiovascular effects of alcohol), Lieber (1985; on the hepatic effects of ethanol), Harper (1998; on the toxicity of alcohol on the brain), and Pohorecky and Brick (1988; on the pharmacology of ethanol). In addition, several scientific and medical journals are devoted to the study of alcohol dependence, such as *Alcohol*, *Alcohol and Alcoholism*, and *Journal of Studies on Alcohol.*

Because the toxic effects of alcohol on humans are well characterized after centuries of experience, the experimental literature on ethanol focuses on specific endpoints, rather than the numerous simultaneous endpoints examined in regulatory toxicology protocols for repeat dosing. These specific endpoints (such as liver toxicity, immunotoxicity, neurotoxicity, etc.) are not addressed in the robust summary for ethanol. However, that experimental literature, in combination with the human health literature and the 90-day studies included in the robust summary, constitutes a very large toxicity database for ethanol. No additional testing is proposed at this time.

e. Reproductive toxicity

The robust summary for ethanol includes four studies, two using mice (CD-l and Swiss Webster strains) and two using rats (Holtzmann). All of the experiments supplied ethanol to animals in drinking water or liquid diet. Three examined fertility in males or females in one-generation designs, while the fourth, conducted on behalf of the National Toxicology Program, assessed fertility in both sexes using a two-generation, continuous reproduction protocol. Numerous other investigations, using both *in vivo* and *in vitro* systems, focus on specific effects of ethanol on the reproductive system or on conception (e.g., Cebral *et al.*, 1997; Anderson *et al.*, 1987, 1985) . The effects of ethanol on fertility has been reviewed by several authors, including Galaver *et al.* (1987) for the International Commission for Protection Against Environmental Mutagens and Carcinogens, IARC (1988), and Anderson *et al.* (1983). No additional testing is proposed.

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f. Developmental toxicity

Ethanol (specifically, alcohol abuse) was recognized as a human teratogen well before experimental studies in animals were undertaken. Fetal alcohol syndrome (FAS) has been extensively studied: for example, a search of the MEDLINE database for studies in English on fetal alcohol syndrome elicits more than 1,500 bibliographic citations. Hundreds of studies using laboratory animals have explored the physical, neurologic, and neurobehavioral abnormalities caused by in *utero* exposure to ethanol, using in vivo and *in* vitro models and acute and chronic exposures. Recent reviews of teratogenicity of ethanol in lab animals include Guerri (1996), Becker *et al.* (1996), Zajac and Abel (1992), and Webster and Ritichie (1991). IARC (1988) also reviewed the developmental toxicity of ethanol towards humans and lab animals.

The robust summary for ethanol describes six experiments in which pregnant mice (five strains) or rats (Sprague-Dawley) were given ethanol during gestation (and in some cases, before mating) by gavage, inhalation, or in liquid diets. These give a good overview of the database pertaining to chronic (*i.* e., at least several days) gestational exposure. In light of the very large database on developmental toxicity of ethanol, no further testing is proposed.

Robust Summary for Ethanol

The robust summary for ethanol, prepared using EPA's HPV Tracker software, is submitted electronically.

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